Review

The microbiota-gut-brain axis and Alzheimer disease. From dysbiosis to neurodegeneration: focus on the central nervous system glial cells

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Abstract: The microbiota-gut system can be thought of as a single unit that interacts with the brain via the so-called two-ways microbiota-gut-brain axis. Through this axis, a constant dialogue mediated by the several products originating from the microbiota guarantees a physiological development and shaping of the gut and the brain. In the present review will be described the modalities through which the microbiota and gut control each other, and the main microbiota products conditioning both local and brain homeostasis.
Much evidence has accumulated over the past decade in favor of a significant association between dysbiosis, neuroinflammation and neurodegeneration. Presently, the pathogenetic mechanisms triggered by molecules produced by the altered microbiota, also responsible for the onset and evolution of Alzheimer Disease will be described. Our attention will be focused on the role of astrocytes and microglia. Numerous studies have progressively demonstrated how these glial cells are important to ensure an adequate environment for neuronal activity in healthy conditions. Furthermore, it is becoming evident how both cell types can mediate the onset of neuroinflammation and lead to neurodegeneration when subjected to pathological stimuli. Based on this information, the role of major microbiota products in shifting the activation profiles of astrocytes and microglia from a healthy to a diseased state will be discussed focussing on Alzheimer Disease pathogenesis.

Keywords: amyloid-β; endotoxin; short chain fatty acids; clasmatodendrosis; cytokines; neurovascular unit; vagus nerve; Toll-like Receptor 4

1. The MICROBIOTA-GUT-BRAIN axis

The gut and its microbiota represent the largest absorption organ, and the largest reservoir of microbes in the human body, respectively. The microbiota consists of almost 1014 microorganisms that are mainly bacteria. These are the Gram-positive *Firmicutes* (51% of the population), most of which are *Lactobacilli*, and the Gram-negative *Bacteroidetes* (48%). Physiologically and pathologically the gut and its microbiota can be considered a single system (microbiota-gut) whose interactions give rise to responses that affect the functions in organs and systems of the whole organism. Among the systems involved, the central nervous system (CNS) is in constant communication with the microbiota-gut, in the so-called two-ways microbiota-gut-brain axis. This interaction involves distant and local networks through neural, immunological, metabolic, and hormonal signaling pathways [1], thus dysfunction at any step of the axis may affect all the other components. It has been shown that brain diseases alter the neurochemistry of the enteric nervous systems (ENS), the functioning of the immune system (IS) and the microbiota itself, using top-to-bottom

directional pathways [2,3,4]. In addition, several bottom-to-top directional pathways, activated by microbiota products, are necessary for the correct development and physiological functioning of the brain [5]. Changes in microbiota composition, the dysbiosis, contribute to several neurodegenerative disorders such as Alzheimer Disease (AD) [2,5-8], Parkinson's Disease (PD) [9], multiple sclerosis (MS) [10], amyotrophic lateral sclerosis [11].

1.1 The microbiota-gut as a unique system

As previously mentioned, the microbiota-gut can be considered as a single unit with respect to the microbiota-gut-brain axis. Any effect produced in the CNS depends on activities resulting from the microbiota and gut continuous interaction. In this interplay, the microbiota has a key role by producing different types of molecules, which are expressed on the surface of the microorganism or secreted. Of note, also the molecules present on the surface can be physiologically secreted as outer membrane vesicles [12]. The contribution of each bacterial species to the integrity/dysfunction of the gut-brain axis is only partially known.

The Gram-positive bacteria produce short chain fatty acids (SCFA) that exert a trophic action on the enterocytes, favour the Treg lymphocyte conversion and, crossing the blood brain barrier (BBB), exert anti-inflammatory activity in the brain; Gram-positive bacteria also metabolize glutamate to ◎-aminobutyric acid (GABA) [13] and support the expression of anti-inflammatory Toll-like receptors (TLR) 2 and 9, favouring the Treg lymphocyte conversion [14]. *Lactobacilli* have been demonstrated to generate tryptophan metabolites that stimulate the type-3 innate lymphoid cells (ILC3) to produce interleukin 22 (IL22) (see below). It has also been reported that the microbiota modulates neuronal activity through the production of neurotransmitters or the modulation of host neurotransmitter catabolism (for references see [15]. Different bacteria strains produce different neurotransmitters such as catecholamines, GABA, serotonin, glutamate. Microbiota-derived metabolites can also directly affect the host immune system, which, in turn, can influence the behaviour of glial cells in both ENS and CNS. Consequently, modifications of the microbiota composition in terms of strains can have impact on the host physiology, at both local and systemic level [15].

The Gram-negative bacteria are the main producers of Aβ prion-like proteins (i.e., a-synuclein) and lipopolysaccharides (LPS) and select pro-inflammatory TLR4 [8,13,16,17]. Among the Gram-negative, *B. subtilis* and *E. choli* are great producers of Aβ and LPS.

In turn, the gut controls the microbiota though several cell populations. The goblet cells produce mucins and, together with the enterocytes, molecules with antimicrobial properties; the microfold M and dendritic cells convey luminal antigens to the Payer patches and neighbour lymphoid nodes [18]; the ILC3 which produce IL22 play a major role to guarantee the epithelium integrity preventing systemic dissemination of commensal and pathogenic microbes [19,20]. Finally, the enteric glial cells actively participate in the maintenance of local homeostasis playing roles in neurons-to-IS communication, intestinal barrier (IB) integrity, neurotransmitter processing and neuroinflammation [21]. Interestingly, one of the most important targets of the microbiota-derived metabolites are the enteroendocrine cells (EECs), which comprise only 1% of the epithelium, but collectively form the largest endocrine system in mammals. These "primed" cells, acting as chemical sensors, have the capability to trigger further changes in other cells in the microbiota-gut system (e.g., primary afferent neurons and enteric glial cells) by releasing vesicles containing hormones, neurotransmitters and other uncharacterized second messengers [22].

Thus, the ability of the intestinal cells to handle molecules of bacterial origin explains why and how these molecules have access to the entire organism up to the brain, causing beneficial or pathological effects depending on their properties [23].

1.2 Microbiota-gut system, from dysbiosis to neurodegeneration

Changes in microbiota composition, the dysbiosis, might cause an excessive production of aggressive molecules, IB and BBB dysfunctions and the development of several gut and brain disorders [2,5,8,12,24]. The microbiota changes spontaneously through life and during aging the ratio between Gram-positive and Gram-negative bacteria inverts. It remains to be determined how dysbiosis contributes to neurodegeneration and/or vice versa. Though, the accumulated evidence demonstrates a significant association between dysbiosis and the development of neuroinflammation and neurodegeneration. Indeed, in several neurodegenerative diseases a consistent decrease of SCFA [25], high levels of Aβ and LPS (AD brain [26] and low levels of GABA have been reported [13]. LPS of microbiota-gut origin as well as infiltrating lymphocytes were found in the brains of Alzheimer patients [27,28]. Finally, dysbiosis has been found in patients affected by neurodegeneration [29-31] while APP/PS1 transgenic mice, that overproduce Aβ, harbour altered microbiota [32].

Literature data also show that probiotic supplementation rich in Gram-positive bacteria improves cognition in patients with AD [33] and the diet has been proved to prevent or reduce the risk to develop cognitive impairment in animals and humans [34-37]. In the animal, long-lasting high-fat diet induces cerebral amyloidosis, commensurate with dietary-induced hyperlipidaemia and with increase of chylomicrons (CM) concentration; starvation reduces the formation of Aβ in the intestine [38,39]. In humans, high-fat and cholesterol-rich diets increase AD risk [40] while Mediterranean and Asian diets may protect against cognitive decline and delay the onset of AD [35].

Attempts have been made to identify those alterations in the microbiota-gut system that could predispose or favour the development of neuroinflammation, and neurodegeneration. An interesting hypothesis on this topic has recently been formulated (see the insert). It that underlines the main role of the microbiota-gut system and indicate it as the privileged target for interventions aimed to prevent the appearance of neurodegenerative diseases or, at least, to slow down their evolution.

2. The DYSBIOSIS and the ALZHEIMER DISEASE

Increased lifespan has resulted in increased frequency of age-related diseases, including AD, the most common type of dementia accounting for more than 65% of all dementia cases. AD currently affects approximately 40 million aged people in Western countries. The increased life expectancy in the world population has seen a progressive increment of this type of dementia and it is expected to triplicate in incidence by 2050. Indeed, beyond the familial forms of AD, at relatively early onset, the idiopathic and most common forms of AD have late onset and are indicated with the acronym LOAD (late onset AD).
However, since 2010, it was raised the question if AD depends on aging or, instead, the late age allows the disease to become clinically manifest as the result of accumulation of stress factors through the lifetime [41]. Among the identified factors, alterations in gut microbiota, and subsequent inflammatory processes have been considered responsible for the later (15-20) appearance of neurodegeneration [42,43].
AD is a neurodegenerative pathology characterized by a slow, irreversible decline of the cognitive functions that affects different brain regions. To date, there are no effective pharmacologic agents to prevent or slow-down the disease progression.
The histopathological hallmark of AD is the accumulation in the brain of misfolded Aβ peptides that organize in fibrils and deposit in plaques [44,45]. The origin of Aβ has not been clearly established. The literature has mainly focused on the Aβ produced in the

brain [46] hypothesizing that Aβ is formed in brain neurons and, with cholesterol and ApoE derived from astrocytes or via the BBB, is embedded in vesicles for further processing and clearance. However, many mechanisms involved in this metabolic pathway are still not well understood, such as whether Aβ plasma concentrations or the ApoE alleles correlate and influence AD-risk, or how plasma phospholipids are involved and why dietary factors seem to have protective effects [35].

Although findings showing a relation between AD and dysbiosis are several, the mechanistic link between the gut and brain in AD progression is limited [47]. Studies have shown that, besides the brain, the microbiota-gut system is a site of Aβ production. It has been reported that the enterocytes contain substantial amounts of Aβ [39,48] and its presence is regulated by diet and intestinal microbiota [17,24]. Gram-negative bacteria are a significant source of Aβ and of LPS, and the increased levels of these molecules found in AD brain plaques are related to dysbiosis, thus Gram-negative bacteria are likely to be involved in the pathogenesis of neurodegeneration [12,49]. The question could be: how Aβ and LPS reach the brain? Two different routes have been considered. The first begins from the enterocytes, where Aβ and LPS are integrated in CM containing ApoE proteins [12,17,48] and, through the blood stream reach the brain [44,50]. For Aβ a second route of diffusion has been postulated: because Aβ belongs to the prion-like proteins, it could arrive to the brain via a neuron-to-neuron retrograde transport from the ENS to the brain through the vagus nerve [24,51,52]. This retrograde neuronal pathway was already described for ◎-synuclein in Parkinson disease [8,51]. When Aβ reaches the brain, local chaperonins, and the receptors for advanced glycosylation products (RAGE) are debuted to their clearance through the BBB. Overload or defective clearance of Aβ may cause its accumulation, favour fibrils organization and their deposition. Similarly, high level of LPS increase the BBB permeability, enter the brain, and activate several inflammatory pathways [12,53]. Recently, some amino acids, such as isoleucine and phenylalanine have also gained importance in AD pathogenesis. It has been reported that these amino acids drive neuroinflammation during AD progression through stimulating differentiation and proliferation of pro-inflammatory T helper 1 (Th1) cells [54]. Interestingly, administration of sodium oligomannate (GV-971), a mixture of oligosaccharides, has been shown to reduce the levels of these amino acids in the blood and brain of AD animal models and promote a consistent cognition improvement in mild to moderate AD in humans [54]. Significantly, this probiotic has completed the first Phase III clinical trial in China [55]. In 2020 the U.S. Food and Drug Administration (FDA) gave a formal nod to commence a Phase III clinical trial in the United States to test the drug GV-971 on patients with AD.

3. The MICROBIOTA and the CENTRAL NERVOUS SYSTEM GLIAL CELLS

The contribution of the different bacterial strains to the integrity and dysfunction of the microbiota-gut-brain axis is not completely known. Much remains to be explored about microbial taxa that regulate microglia and astrocyte functions. Microglia and astrocytes can have simultaneously multiple profiles of activation, which can represent the extremes of a continuous spectrum of reactive profiles [56]. The mechanisms regulating these diverse functional properties remain unknown, but evidence suggests that environmental cues, such as those deriving from the microbiota, are important.

Some brain areas, such as the cortex, hippocampus and amygdala, are particularly susceptible to the products of the microbiota [5] and these areas correspond to those primarily altered in AD. Although brain diseases were traditionally attributed solely to malfunctioning of neurons, it is becoming more and more evident that proper interplays among neurons, astrocytes, microglia with peripherally derived cells and molecules are of fundamental importance for the physio-pathological organization of the brain [57].

The following paragraphs of the review will delineate the current knowledge on how microbiota regulates the physiological and pathological functions of astrocytes and microglia, to assess how these interactions can influence the disease state and its progression.

3.1 How the microbiota shapes astrocytes

Astrocytes, the most numerous glia cells of the CNS, are endowed of many housekeeping functions and help maintaining the brain in healthy conditions [58]. Healthy astrocytes with their processes envelop synapses and are indispensable for synaptogenesis, synaptic pruning, maintenance and maturation of synapses, release of gliotransmitters and neurotransmitter homeostasis [59,60]. In addition, astrocytes control the synaptic levels of GABA and glutamate, mediating the functions of the so-called tripartite synapses, with neurons and microglia [61], and contributing to synaptic activity and memory formation [62]. Healthy astrocytes form a cellular network, interconnected through gap junctions, which regulate the cellular homeostasis of water and ions. Astrocytes are an integral part of the BBB, of the neurovascular unit (NVU), and of the glymphatic system and regulate neurovascular coupling, vascular tone and blood flow [58,61,63,64]. Indeed, astrocytes end feet that surround the walls of the vessels are main components of the NVU and, together with perivascular microglia and macrophages, survey the influx end efflux of molecules [65]. Disruption of the NVU is associated with vascular dementia [66] and increased permeability of the BBB has been observed in subjects with mild cognitive impairment [67], likely contributing to the early stages of AD [68], as also shown in animal models of the disease [69-71]. Astrocytes, in their activated form (A1 astrocytes) express and release cytokines that modify the permeability of the BBB [72], and activation (astrogliosis) of perivascular astrocytes causes loss of aquaporin4 (AQP4) polarization, and may cause vascular and glymphatic dysregulation and BBB disorganization, considered among the first steps in AD pathogenesis [70,73,74]. As a matter of fact, the glymphatic system is impaired during aging [75] and its dysfunction is involved in many neurodegenerative disorders, particularly those in which accumulation of extracellular waste is an important characteristic. Furthermore, aged astrocytes undergo a morphological modification named clasmatodendrosis [76,77], which consists of fragmentation and shortening of astrocytes distal processes. Clasmatodendrotic alterations of astrocytes are associated with changes in cell function [78], which can compromise the integrity of the BBB [79]. The clasmatodendrotic modification of astrocytes during aging [80] and ischemia [77] may represent one of the causes not only of vascular, but also of glymphatic dysfunction. Therefore, clasmatodendrosis can hamper astrocyte-mediated Aβ clearance from neurons and increase fibrillar Aβ deposition [81-82]. The deposition of high quantities of fibrillar Aβ modifies the interactions between astrocytes and neurons [82], possibly decreasing Aβ peptide disposal to the circulating system, and, consequently, increasing Aβ deposition in brain parenchyma [83] that may play a significant role in neuronal damage. In mouse models of AD, the impairment of Aβ clearance increases neurodegeneration [84].

It has been demonstrated that endothelial cells of germ-free (GF) mice have decreased expression of occludin and claudin-5, with consequent disorganization of tight junctions and increased permeability of the colonic barrier [85] and of the BBB [86]. These data indicate that products of the microbiota, mainly butyrate, maintain the integrity of these barriers [87]. Indeed, in GF mice the integrity of BBB can be restored by recolonization of the gut by microbiota, which increases the expression of tight junction proteins and restoration of the BBB [86], or by supplementation with SCFAs [88]. Furthermore, molecules of bacterial origin such as LPS induce the transcription of proinflammatory and cytotoxic pathways in astrocytes [89] and breakdown of inter-cellular tight junctions [90], further structural and functional alterations of the BBB. The BBB seals during early postnatal life, and, separating the CNS from the periphery, creates a milieu that is required for proper functional activity of neurons and neuronal circuits [91,92]. Therefore, alterations of the BBB, as those caused by dysbiosis, allowing the passage of proinflammatory factors, of immune cells from the periphery and of peptides such as Aβ, modify the composition of the cerebral milieu and the homeostasis of brain cells. In a further study, it has been shown that during treatment with antibiotics, while in the hippocampus the expression of tight junction proteins decreases, in the amygdala it increases [93]. This region-specific differentiation of BBB permeability can possibly result in differential passages of molecules in the various regions of the brain, with differential effects. It is still to be understood the

causes of the diverse spatial responses present in different brain areas to peripheral stimuli.

Disruption of BBB, NVU and of the glymphatic system causes reduction of transport and inefficient removal of toxic substances, which can accumulate in brain parenchyma, implementing a vicious circle of neuroinflammation and tissue damage [72,74,94–98]. In this respect it should be pointed out that the glymphatic system facilitates the clearance of interstitial Aβ and tau [99], and the impairment of all these mechanisms may decrease Aβ clearance [75,100,101] and increase Aβ extracellular levels. All these data suggest that modifications of astrocytes functionality caused by dysbiosis is responsible for microlesions of the NVU and of the glymphatic system, decreasing the disposal of Aβ peptides in the brain parenchyma, and increasing the risk of amyloid plaque formation [99].

3.2 Microbial products that shape astrocytes

While it is known that several factors within or outside the CNS cause activation of astrocytes from their healthy state, much remains to be explored with regards to microbial taxa that finely regulate astrocyte functions. Microbial-derived products and metabolic byproducts (SCFAs) activate distinct immune pathways in the host. The gut microbiota is able to modulate the activity of astrocytes metabolizing dietary tryptophan to produce natural ligands for aryl hydrocarbon receptors (AHRs), including indole-3-aldehyde and indole-3-propionic acid, that bind to astrocyte AHR [20,88,102]. It has been demonstrated that indole-3-aldehyde treatment reduces expression of proinflammatory factors [88]. Furthermore, upregulation of AHRs in astrocytes results in anti-inflammatory activity trough the Interferon-I (IFN-I) signalling [88]. It appears that IFN-I works with microbiota-produced dietary tryptophan to activate AHRs in astrocytes and to suppress inflammatory mechanisms [88]. Collectively, these findings suggest that microbial metabolites of dietary tryptophan can modulate the inflammatory status of astrocytes, with important consequences for neuroinflammation. The involvement of peripheral bacteria in brain health and disease conditions can be also envisaged by other data. Indeed, *Porphyromonas gingivalis*, one of the most common Gram negative bacteria in oral chronic inflammatory diseases, activate astrocytes via TLR4, thus increasing cytokines production and contributing to the inflammatory lesions [103,104].

3.3 How the gut microbiota shapes microglia

Microglia are myeloid cells that invade the brain during early development and have dynamic roles in the coordination of responses between the immunity system and cognitive functions [105-114]. Microglia are the primary immune cells of the CNS, and, being active responders to peripheral stimuli, the standard notion that the brain is an "immune privileged" organ is rapidly changing [115]. Indeed, various pathological stimuli cause rapid recruitment of microglia to the site of injury, resulting in a resident innate immune response [116-118]. Dysfunction of microglia has been described in many CNS disorders such as AD [119], frontotemporal dementia [120-121] and PD [122]. The ability of microglia to maintain their protective role by clearing dying neurons [123] decreases considerably in a proinflammatory context [124]. For instance, in APP-SL70 mice, a transgenic model of AD, microglia phagocytic activity inversely correlates with Aβ plaque deposition and aging [125].

In physiological conditions, microglia are highly dynamic and mobile cells and their highly mobile projections are necessary to sense the domains of neighboring microglia cells to avoid their spatial overlap [126-127]. In the absence of microbiota, such as in GF or specific pathogen free (SPF) mice, microglia morphology is severely altered, and the cells have longer, very mobile, hyperramified projections [128-130], which enter in physical contacts with projections of adjacent cells and partially overlap in the spatial domains of neighboring microglia [128]. In GF or SPF mice treated with antibiotics, microglia phenotype returns to normal [128]. Which might be the consequences of the hyperramified microglia phenotype in microbiota-free animals is not clear, although a recent research

shows that microglia prune synapses in a microbiota-dependent manner [131], which indicates a possible functional consequence of alterations of microglia projections.

As discussed above, an emerging hypothesis is that the microbiota influences AD pathology increasing Aβ production in the gut, which may cause increased Aβ deposition in the brain, Aβ plaque formation and activation of microglia. Activated microglia migrate to the sites of Aβ plaques, interact with Aβ deposits and regulate Aβ levels in the brain [132-133]. Germ Free APP/PS1 mice (a transgenic model of AD) have drastic reduction of Aβ levels and of compact Aβ plaques, as well as decrease of IBA1 positive microglia, in comparison to APP/PS1 mice with normal microbiota [32]. Therefore, it appears that signals from the microbiota delineate microglia morphology and functionality, and dysbiosis causes microglia dysfunctionality. In particular, Erny and coworkers [128] demonstrated that the microbiota is important for maturation and maintenance of microglia in proper steady-state physiological conditions, ready to display a rapid response to damaging stimuli. Reconstruction of microbiota reverses, although not completely, microglia cell morphology [128]. Nevertheless, how the gut microbiota can control microglia maturation at such distant sites such as the CNS remains to be unraveled.

Despite the early description of diverse phenotypes of microglia by Del Rio-Hortega (1919) [134], the common and long-lasting idea that microglia can undergo a simple, univocal process of activation followed by deactivation, has survived for several decades. This idea has undoubtedly prevented the understanding and recognition of the diversity of microglia phenotypical modifications in response to external stimuli, and the extraordinary plasticity of these cells [135]. In the last decade it has become clear that microglia respond to noxious stimuli integrating multifarious inputs and their responses can be opposite and, depending upon the stimulus, can induce neuroprotective or neurotoxic effects. Indeed, the original definition of microglia activation has been revised on the demonstration that microglia can assume at least two different phenotypic forms, M1 and M2 [136-138]. It is now clear that M1 and M2 represent the two extremes of an entire spectrum of activation patterns. M1 microglia express proinflammatory cytokines such as IL-6 and TNF-α. M2 microglia express high levels of arginase-1 (Arg-1) and IL-10 [137-138]. The M2 phenotype is thus more active in phagocytosis of apoptotic or dying neurons to prevent secondary inflammatory mechanisms and promote tissue regeneration [57,139-149]. Furthermore, phagocytic microglia are classified into M2a, M2b and M2c in the absence of inflammation and induce a Th2-like response [141]. Recently, it has been demonstrated in post mortem studies that microglia show higher heterogeneity in humans than in primates and rodents [142-142]. Not only species differences, but also region-specificity characterize microglia diversification. Indeed, it has been demonstrated that microglia have age- and region-dependent transcriptional identities in a region-specific way [144]. For instance, in the hippocampus microglia have a high "immune-vigilant" phenotype which can be responsible for the higher microglia activation in response to Aβ plaque formation, giving rise to a harmful chronic inflammatory response [144]). Hart et al. (2012) [145] showed a further regional difference between microglia located in the white matter versus microglia located in the grey matter [145]. In a different study it was demonstrated that hippocampal microglia display lower expression of many proteins, among which CXCR3 [146], a receptor involved in neuron-microglia communication, in microglia recruitment, neuronal reorganization [147], and in microglia activation during demyelination [148]. Therefore, decreased levels of CXCR3 receptor, and other proteins in AD-vulnerable brain regions such as the hippocampus, may impair microglia response and recruitment. Consequently, region-specific variations (both increase or decrease) in gene expression may be implicated in the progression of neurodegenerative diseases [149].

3.4 Microbial products that shape microglia

Bacterial-produced molecules, such as LPS, peptidoglycans and PAMPs (Pathogen Associated Molecular Patterns) [150] can cross both the IB and the BBB [151-152], and can reach the brain parenchyma where they can be recognized by TLR4 expressed on microglia, which plays an important role in neuroinflammation [153]. Further, stimulation of TLR2

by fibrillar Aβ activates microglia into a more pro-inflammatory profile, with detrimental effects on AD pathology [154]. Nevertheless, although microglia actively maintain their protective role during normal aging [118,155–157] by clearing dying neurons [123], their capability is considerably impaired in acute pro-inflammatory contexts. Furthermore, sustained activation of microglia can increase Aβ deposition and phagocytosis of healthy neurons [158-164].

Therefore, the microbiota shapes the brain innate IS, conditioning the maturation and function of microglia [128], which, in turn, has a dynamic role in coordinating the responses between the IS and cognitive functions [107,108,114], microglia survey brain parenchyma, maintain microenvironmental tissue homeostasis [117], perform pruning of synapses or phagocytosis of apoptotic neurons and debris, and maintain astrocyte functions [107,108,114]. The regulation of neuronal activity (activation, inhibition, potentiation, or depression) has been viewed for over a century as an exclusive prerogative of neurons themselves. Nevertheless, recent data demonstrate that even microglia can be involved in this process, acting similarly to inhibitory neurons to suppress excessive neuronal activity [165-170], at least in the striatum, but possibly also in other brain regions. It is thus conceivable that modifications of microglia phenotype during their activation (such as downregulation of P2RY12 and other receptors) can contribute to pathological dysfunctions of neuron excitability and consequent behavioural alterations typical of neurodegenerative disorders such as AD [171-174].

The physiological interactions of M1/M2 can be compromised in brain pathologies, and microglia often actively participates in disease progression. In AD, microglia, activated by danger signals such as ATP released from dying neurons, retract their branched processes, round up, produce IL-1β, TNF-α, ROS and NO, thus contributing to amplification of inflammation and neurodegeneration. Indeed, the possibility to control and modify the microglia phenotype represents a challenge to contrast this disease. The host microbiota is an essential environmental factor that shapes the brain innate IS, and particularly the maturation and function of microglia [128].

Many hypotheses have been postulated to explain how the microbiota can regulate microglia. I) SCFAs generated by the microbiota, can cross the BBB. Once in the CNS, SCFAs target microglia and regulate their function or maturation. II) Immune cells expressing receptors for SCFAs, after interacting with SCFAs, can migrate to the brain via the BBB. III) Before the expression of SCFAs-recognizing receptors, other metabolites or compounds called microbe-associated molecular patterns (MAMPs), produced by the microbiota, can cross the BBB and target microglia to regulate their function or maturation. IV) Peripheral macrophages that can recognize MAMPs released by the gut microbiota can migrate to the brain and cross the BBB. V) Finally, the gut microbiota can communicate directly with the CNS resident microglia through the vagus nerve [175]. The vagus nerve senses changes of proinflammatory cytokines caused by inflammation in the gut and through its afferent fibers sends information to the CNS and influences microglia and inflammatory mechanisms [176]. Most of the above mechanisms take advantage of the disruption of the BBB or the glymphatic system, described in the previous paragraph (see Figure 1).

Furthermore, epigenetic mechanisms can shape the identity of macrophages during development, but local microenvironment within and outside the brain can additionally reprogram the genetic imprint [128,177,178]. Although little is known on the epigenetic mechanisms that control the function/activation of microglia, prenatal ablation of histone deacetylases1/2 (HDAC1/2) impairs microglia development, while it has no effect on microglia homeostasis in adult mice [179]. Interestingly, in a mouse model of AD, deficiency of HDAC1/2 in microglia increases amyloid phagocytosis, resulting in decreased Aβ load, and amelioration of cognitive impairment [179]. It appears therefore that epigenetic factors, which can have different outputs whether during development or in adulthood, affect microglia maturation, homeostasis and activation in a differential manner. The gut-

microbiota can affect epigenetic modifications throughout the entire lifespan, as has been demonstrated in diabetes and obesity [180], but possibly also in AD.

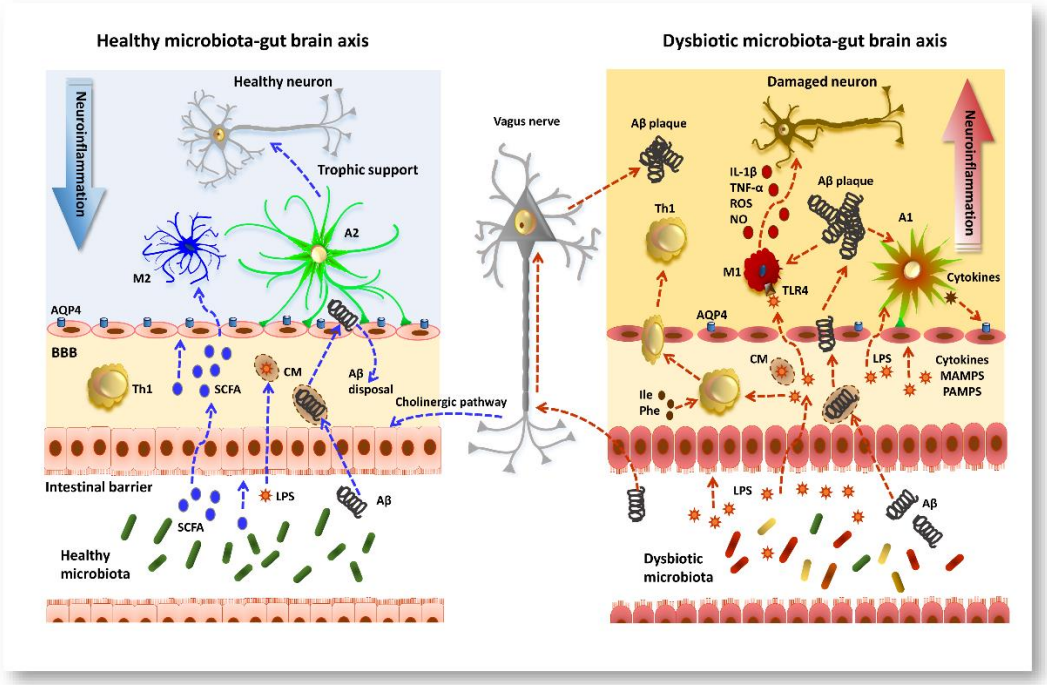

Figure 1. Schematic representation of the bottom-to-top regulation of neuroinflammation in AD pathogenesis. *Left panel*. In healthy conditions, the microbiota gut–brain axis modulates key processes, including immune cell maturation and maintenance of the gut epithelium. SCFAs produced by the gut microbiota cross the IB and, via the circulatory system, reach and cross the blood-brain barrier. Once in the brain parenchyma, SCFAs target microglia and regulate their functions. The gut microbiota is one of the main producers of Aβ peptide and LPS, which integrate in CM and cross the BBB. Aβ is readily retro transported to the circulatory system for its disposal. Blue lines represent known beneficial pathways of microbiota. *Right panel.* Microbiota overproduction of LPS and cytokines causes modification of the permeability of the gut epithelium, of the NVU and of the glymphatic system. Gut microbiota production of SCFA is reduced in AD, while the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, as well as MAMPA and PAMPS is increased. These factors translocate to the brain where they modulate microglia via TLR4, and activated M1 microglia release IL-1β, TNF-α, ROS, NO that cause neuronal damage. In addition, proinflammatory cytokines cause activation of astrocytes (A1), which release cytokines that in turn decrease AQP4 expression and modify NVU permeability. Peripheral Th1, activated by isoleucine (Ile) and phenylalanine (Phe) produced by microbiota, can recognize the bacterial metabolites or MAMPs and migrate to the brain via the damaged BBB. Aβ peptide produced by the microbiota can easily cross the IB or be retrogradely transported to the brain via the vagus nerve. Since disposal of Aβ peptide is impaired by the damage to the BBB, Aβ peptide precipitate to form plaques, which further worsen microgliosis and astrogliosis, increasing the severity of AD pathology. Red lines indicate the bottom-to-top damaging pathways so far demonstrated.

Recently it has been shown in APP/PS1 mice that a dynamic shift of gut microbiota composition is significantly correlated with the increase of Th1 cells infiltration into the brain [54]. Ablation of the gut microbiota by antibiotics blocks Th1 cells infiltration and M1 microglia activation. These findings highlight that gut microbiota is a driving factor in promoting Th1/M1 microglia neuroinflammation in AD progression [54].

4. Conclusions

In this review we have summarized the role of the microbiota gut–brain axis as an integral part of the pathogenesis of AD. Indeed, the two-ways interactions among the intestinal microbiota, the peripheral immune system and the CNS are essential for the maintenance of host health, and their dysregulation can be one of the initiating factors in multifactorial

chronic neuroinflammatory diseases, such as AD. Neuronal pathways, hormones, microbial molecules, and metabolites are all involved in the signalling between these two regions. Although the causes of AD are still not clear, and no curative treatments are available, experimental, and clinical data collected strongly address the research versus preventive approaches aimed at reducing Aβ production and/or inhibiting self-assembly of amyloidogenic peptides. Modification of the composition of the microbiota destroys the bottom-to-top communication that ultimately influences brain motor, sensory, and cognitive functions, maintains brain homeostasis and/or contributes to the onset of pathological conditions. Elucidating the interplay between the gut microbiota and the central nervous system, and the role of microbiota in neuroinflammation will lead to a better understanding of many neurodegenerative diseases pathogenesis. Furthermore, a deeper knowledge of these interactions may lead to new therapeutic approaches through which neuroinflammation can be dampened acting indirectly through the microbiota-gut-brain axis.

INSERT

The endotoxin hypothesis. This hypothesis rests on accumulated evidence highlighting the role of LPS in the pathogenesis of neurodegenerative diseases [12]. Known since the end of the XIX century, endotoxins determine inflammation and toxicity [181]. Endotoxins are common component of the Gram-negative plasma membrane, located in the external layer. Endotoxins can be released following bacterial death or as external membrane vesicles. High levels of Gram-negative bacteria containing and producing endotoxins, are found in the lower mammalian intestine [182].

The endotoxins, once released, manifest significant differences in their biological activity based on the properties of the lipophilic lipid A portion. In particular, the presence of 6 acyl chains makes the molecule particularly aggressive [183]. Several species of Gram-negative bacteria produce LPS but the greater producer of the 6-acyl chain is E. coli which is also a great producer of Aβ [12]. All the endotoxins bind to the MD2/TLR4 receptor (a complex of myeloid differentiation factor 2 and toll-like receptor 4), however, while the 6 acyl chains variant strongly activates it, inducing an intense inflammatory response, the 4 or 5 acyl chains act as antagonists on the same receptor. Endotoxins, for their chemical properties, cross the plasma membranes, enter the intestinal cells and, bound to albumin or HDL or chylomicrons, reach the blood stream and the brain. Small amounts of plasmatic endotoxin are detected in all healthy humans; however, higher levels of these molecules have been constantly found in PD, AD, and motor neuron diseases. Indeed, high levels of endotoxin in the gut and brain have been shown to impair the IB and BBB integrity because of local inflammation and to favour the accumulation of other potential toxic molecules such as Aβ, α-synuclein and some amino acids [54]. Ultimately, high levels of endotoxin also promote the production or aggregation of Aβ, tau protein and α-synuclein in the brain [12].

In the brain, endotoxins target microglia selecting the pro-inflammatory M1 phenotype, and astrocytes. M1 and astrocytes produce high quantity of iNOS and cytokines via activation of the TLR4 and phagocyte death neurons or even stressed-but-viable neurons through the mechanism of phagoptosis [12,184]. It has not yet been established whether endotoxins prime microglia to neurodegenerative stimuli or vice versa [185]. There is clinical evidence that systemic inflammation accelerates cognitive decline in AD patients. In summary, it is reasonable to assume that any intervention aimed at preventing or treating dysbiosis (reducing the production of toxic molecules) could interrupt or at least slow down the vicious circle endotoxins-neuroinflammation-neurodegeneration [12].

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List of abbreviations

Aβ: amyloid-beta

AHRs: Aryl Hydrocarbon Receptors
AQP4: aquaporin4
BBB: Blood Brain Barrier
CM: chylomicrons
CNS: central Nervous System
CXCR3: CXC chemokine receptors3
EECs: Entero-Endocrine cells
ENS: Enteric Nervous System
HDAC1/2: histone deacetylases1/2
IB: Intestinal Barrier
IL: Interleukin
ILC: Innate Lymphoid cells
iNOS: inducible Nitric Oxide Synthase
IS: Immune System
LOAD: Late Onset AD
LPS: lipopolysaccharides
MAMPs: Microbe-Associated Molecular Patterns
NO: nitric Oxide
NVU: Neuro Vascular Unit
PAMPs: Pathogen Associated Molecular Patterns
P2RY12: Purinergic2 ReceptorY12
ROS: Reactive Oxygen Species
SCFA: short chain fatty acid
Th1: T helper 1
TLR: Toll-like receptor
TNF: Tumor Necrosis Factor

References

1. Collins, S.M. A role for the gut microbiota in IBS. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 497–505, doi:10.1038/nrgastro.2014.40.
2. Mayer, E.A.; Tillisch, K. The brain-gut Axis in abdominal pain syndromes. *Annu. Rev. Med.* **2011**, *62*, 381–396, doi:10.1146/annurev-med-012309-103958.
3. Pigrau, M.; Rodiño-Janeiro, B.K.; Casado-Bedmar, M.; Lobo, B.; Vicario, M.; Santos, J.; Alonso-Cotoner, C. The joint power of sex and stress to modulate brain-gut-microbiota axis and intestinal barrier homeostasis: Implications for irritable bowel syndrome. *Neurogastroenterol. Motil.* **2016**, *28*, 463–486, doi:10.1111/nmo.12717.
4. Moloney, R.D.; Johnson, A.C.; O'Mahony, S.M.; Dinan, T.G.; Greenwood-Van Meerveld, B.; Cryan, J.F. Stress and the Microbiota-Gut-Brain Axis in Visceral PaRelevance to Irritable Bowel Syndrome. *CNS Neurosci. Ther.* **2016**, *22*, 102–117, doi: 10.1111/cns.12490.
5. Sharon, G.; Sampson, T.R.; Geschwind, D.H.; Mazmanian, S.K. The Central Nervous System and the Gut Microbiome. *Cell* **2016**, *167*, 915–932, doi: 10.1016/j.cell.2016.10.027.
6. Cattaneo, A.; Cattane, N.; Galluzzi, S.; Provasi, S.; Lopizzo, N.; Festari, C.; Ferrari, C.; Guerra, U.P.; Paghera, B.; Muscio, C.; et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol. Aging* **2017**, *49*, 60–68, doi:10.1016/j.neurobiolaging.2016.08.019.
7. Sun, M.; Ma, K.; Wen, J.; Wang, G.; Zhang, C.; Li, Q.; Bao, X.; Wang, H. A Review of the Brain-Gut-Microbiome Axis and the Potential Role of Microbiota in Alzheimer's Disease. *J. Alzheimers. Dis.* **2020**, *73*, 849–865, doi:10.3233/JAD-190872.
8. Friedland, R.P.; Chapman, M.R. The role of microbial amyloid in neurodegeneration. *PLoS Pathog.* **2017**, *13*, e1006654, doi:10.1371/journal.ppat.1006654.
9. Hopfner, F.; Künstner, A.; Müller, S.H.; Künzel, S.; Zeuner, K.E.; Margraf, N.G.; Deuschl, G.; Baines, J.F.; Kuhlenbäumer, G. Gut microbiota in Parkinson disease in a northern German cohort. *Brain Res.* **2017**, *1667*, 41–45, doi:10.1016/j.brainres.2017.04.019.
10. Kadowaki, A.; Quintana, F.J. The Gut–CNS Axis in Multiple Sclerosis. *Trends Neurosci.* **2020**, *43*, 622–634, doi: 10.1016/j.tins.2020.06.002.

11. Rowin, J.; Xia, Y.; Jung, B.; Sun, J. Gut inflammation and dysbiosis in human motor neuron disease. *Physiol. Rep.* **2017**, *5*, 1–6, doi:10.14814/phy2.13443.
12. Brown, G.C. The endotoxin hypothesis of neurodegeneration. *J. Neuroinflammation* **2019**, *16*, 180, doi:10.1186/s12974-019-1564-7.
13. Pistollato, F.; Cano, S.S.; Elio, I.; Vergara, M.M.; Giampieri, F.; Battino, M. Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. *Nutr. Rev.* **2016**, *74*, 624–634, doi:10.1093/nutrit/nuw023.
14. Hoang, T.K.; He, B.; Wang, T.; Tran, D.Q.; Rhoads, J.M.; Liu, Y. Protective effect of Lactobacillus reuteri DSM 17938 against experimental necrotizing enterocolitis is mediated by toll-like receptor 2. *Am. J. Physiol. - Gastrointest. Liver Physiol.* **2018**, *315*, G231-G240, doi:10.1152/ajpgi.00084.2017.
15. Strandwitz, P. Neurotransmitter modulation by the gut microbiota. *Brain Res.* **2018**, *1693*, 128–133, doi: 10.1016/j.tins.2020.06.002.
16. de Kivit, S.; Tobin, M.C.; Forsyth, C.B.; Keshavarzian, A.; Landay, A.L. Regulation of intestinal immune responses through TLR activation: Implications for pro- and prebiotics. *Front. Immunol.* **2014**, *5*, 60, doi: 10.3389/fimmu.2014.00060.
17. Galloway, S.; Takechi, R.; Nesbit, M.; Pallebage-Gamarallage, M.M.; Lam, V.; Mamo, J.C.L. The differential effects of fatty acids on enterocytic abundance of amyloid-beta. *Lipids Health Dis.* **2019**, *18*, 1–6, doi:10.1186/s12944-019-1162-9.
18. Mabbott, N.A.; Donaldson, D.S.; Ohno, H.; Williams, I.R.; Mahajan, A. Microfold (M) cells: Important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunol.* **2013**, *6*, 666–677, doi: 10.1038/mi.2013.30.
19. Sonnenberg, G.F.; Fouser, L.A.; Artis, D. Border patrol: Regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* **2011**, *12*, 383–390, doi: 10.1038/ni.2025.
20. Zelante, T.; Iannitti, R.G.; Cunha, C.; DeLuca, A.; Giovannini, G.; Pieraccini, G.; Zecchi, R.; D'Angelo, C.; Massi-Benedetti, C.; Fallarino, F.; et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* **2013**, *39*, 372–385, doi:10.1016/j.immuni.2013.08.003.
21. Savidge, T.C.; Newman, P.; Pothoulakis, C.; Ruhl, A.; Neunlist, M.; Bourreille, A.; Hurst, R.; Sofroniew, M. V Enteric Glia Regulate Intestinal Barrier Function and Inflammation Via Release of S-Nitrosoglutathione. *Gastroenterology* **2007**, *132*, 1344–1358, doi:10.1053/j.gastro.2007.01.051.
22. Kuwahara, A.; Matsuda, K.; Kuwahara, Y.; Asano, S.; Inui, T.; Marunaka, Y. Microbiota-gut-brain axis: Enteroendocrine cells and the enteric nervous system form an interface between the microbiota and the central nervous system. *Biomed. Res.* **2020**, *41*, 199–216, doi:10.2220/biomedres.41.199.
23. Kujala, P.; Raymond, C.R.; Romeijn, M.; Godsave, S.F.; van Kasteren, S.I.; Wille, H.; Prusiner, S.B.; Mabbott, N.A.; Peters, P.J. Prion uptake in the gut: Identification of the first uptake and replication sites. *PLoS Pathog.* **2011**, *7*, e1002449, doi:10.1371/journal.ppat.1002449.
24. Willyard, C. How Gut Bacteria Alter the Brain. *Nature* **2021**, *590*, 22–25, doi: 10.1038/d41586-021-00260-3.
25. Unger, M.S.; Marschallinger, J.; Kaindl, J.; Höfling, C.; Rossner, S.; Heneka, M.T.; Van der Linden, A.; Aigner, L. Early Changes in Hippocampal Neurogenesis in Transgenic Mouse Models for Alzheimer's Disease. *Mol. Neurobiol.* **2016**, *53*, 5796–5806, doi:10.1007/s12035-016-0018-9.
26. Zhan, X.; Stamova, B.; Jin, L.W.; Decarli, C.; Phinney, B.; Sharp, F.R. Gram-negative bacterial molecules associate with Alzheimer disease pathology. *Neurology* **2016**, *87*, 2324–2332, doi:10.1212/WNL.0000000000003391.
27. Calvani, R.; Picca, A.; Lo Monaco, M.R.; Landi, F.; Bernabei, R.; Marzetti, E. Of microbes and minds: A narrative review on the second brain aging. *Front. Med.* **2018**, *5*, 1–11, doi:10.3389/fmed.2018.00053.
28. Merlini, M.; Kirabali, T.; Kulic, L.; Nitsch, R.M.; Ferretti, M.T. Extravascular CD3+ T Cells in Brains of Alzheimer Disease Patients Correlate with Tau but Not with Amyloid Pathology: An Immunohistochemical Study. *Neurodegener. Dis.* **2018**, *18*, 49–56, doi:10.1159/000486200.
29. Castelli, V.; D'Angelo, M.; Quintiliani, M.; Benedetti, E.; Cifone, M.G.; Cimini, A. The emerging role of probiotics in

neurodegenerative diseases: New hope for Parkinson's disease? *Neural Regen. Res.* **2021**, *16*, 628–634, doi:10.4103/1673-5374.295270.

30. Kesika, P.; Suganthy, N.; Sivamaruthi, B.S.; Chaiyasut, C. Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. *Life Sci.* **2021**, *264*, 118627, doi:10.1016/j.lfs.2020.118627.
31. Koszewicz, M.; Jaroch, J.; Brzecka, A.; Ejma, M.; Budrewicz, S.; Mikhaleva, L.M.; Muresanu, C.; Schield, P.; Somasundaram, S.G.; Kirkland, C.E.; et al. Dysbiosis is one of the risk factor for stroke and cognitive impairment and potential target for treatment. *Pharmacol. Res.* **2021**, *164*, 105277, doi:10.1016/j.phrs.2020.105277.
32. Harach, T.; Marungruang, N.; Duthilleul, N.; Cheatham, V.; Mc Coy, K.D.; Frisoni, G.; Neher, J.J.; Fåk, F.; Jucker, M.; Lasser, T.; et al. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. *Sci. Rep.* **2017**, *7*, 41802, doi:10.1038/srep41802.
33. Akbari, V.; Hendijani, F. Effects of probiotic supplementation in patients with type 2 diabetes: Systematic review and meta-analysis. *Nutr. Rev.* **2016**, *74*, 774–784, doi:10.1093/nutrit/nuw039.
34. Tremlett, H.; Bauer, K.C.; Appel-Cresswell, S.; Finlay, B.B.; Waubant, E. The gut microbiome in human neurological disease: A review. *Ann. Neurol.* **2017**, *81*, 369–382, doi:10.1002/ana.24901.
35. Casamenti, F.; Stefani, M. Olive polyphenols: new promising agents to combat aging-associated neurodegeneration. *Expert Rev. Neurother.* **2017**, *17*, 345–358, doi: 10.1080/14737175.2017.1245617.
36. Phillips, M.C.L. Fasting as a therapy in neurological disease. *Nutrients* **2019**, *11*, 2501 doi:10.3390/nu11102501.
37. Jensen, N.J.; Wodschow, H.Z.; Nilsson, M.; Rungby, J. Effects of ketone bodies on brain metabolism and function in neurodegenerative diseases. *Int. J. Mol. Sci.* **2020**, *21*, 1–17, doi:10.3390/ijms21228767.
38. Galloway, S.; Pallebage-Gamarallage, M.M.S.; Takechi, R.; Jian, L.; Johnsen, R.D.; Dhaliwal, S.S.; Mamo, J.C.L. Synergistic effects of high fat feeding and apolipoprotein E deletion on enterocytic amyloid-beta abundance. *Lipids Health Dis.* **2008**, *7*, 15, doi:10.1186/1476-511X-7-15.
39. Pallebage-Gamarallage, M.M.; Galloway, S.; Takechi, R.; Dhaliwal, S.; Mamo, J.C.L. Probucol suppresses enterocytic accumulation of Amyloid-β induced by saturated fat and cholesterol feeding. *Lipids* **2012**, *47*, 27–34, doi:10.1007/s11745-011-3595-4.
40. Solfrizzi, V.; D'Introno, A.; Colacicco, A.M.; Capurso, C.; Todarello, O.; Pellicani, V.; Capurso, S.A.; Pietrarossa, G.; Santamato, V.; Capurso, A.; et al. Circulating biomarkers of cognitive decline and dementia. *Clin. Chim. Acta* **2006**, *364*, 91–112, doi:10.1016/j.cca.2005.06.015.
41. Herrup, K. Reimagining Alzheimer's disease - An age-based hypothesis. *J. Neurosci.* **2010**, *30*, 16755–16762, doi:10.1523/JNEUROSCI.4521-10.2010.
42. Fülöp, T.; Itzhaki, R.F.; Balin, B.J.; Miklossy, J.; Barron, A.E. Role of microbes in the development of Alzheimer's disease: State of the Art - An International Symposium Presented at the 2017 IAGG Congress in San Francisco. *Front. Genet.* **2018**, *9*, 1–16, doi:10.3389/fgene.2018.00362.
43. Li, B.; He, Y.; Ma, J.; Huang, P.; Du, J.; Cao, L.; Wang, Y.; Xiao, Q.; Tang, H.; Chen, S. Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. *Alzheimer's Dement.* **2019**, *15*, 1357–1366, doi:10.1016/j.jalz.2019.07.002.
44. Koyama, A.; Okereke, O.I.; Yang, T.; Blacker, D.; Selkoe, D.J.; Grodstein, F. Plasma amyloid-β as a predictor of dementia and cognitive decline: A systematic review and meta-analysis. *Arch. Neurol.* **2012**, *69*, 824–831, doi: 10.1001/archneurol.2011.1841.
45. Jiang, C.; Li, G.; Huang, P.; Liu, Z.; Zhao, B. The Gut Microbiota and Alzheimer's Disease. *J. Alzheimer's Dis.* **2017**, *58*, 1–15, doi: 10.3233/JAD-161141.
46. Hayden, E.Y.; Teplow, D.B. Amyloid β-protein oligomers and Alzheimer's disease. *Alzheimer's Res. Ther.* **2013**, *5*, 60, doi:10.1186/alzrt226.
47. Kowalski, K.; Mulak, A. Brain-gut-microbiota axis in Alzheimer's disease. *J. Neurogastroenterol. Motil.* **2019**, *25*, 48–60, doi:10.5056/jnm18087.

48. Galloway, S.; Takechi, R.; Pallebage-Gamarallage, M.M.S.; Dhaliwal, S.S.; Mamo, J.C.L. Amyloid-b colocalizes with apolipoprotein B in absorptive cells of the small intestine. *Lipids Health Dis.* **2009**, *8*, 46, doi:10.1186/1476-511X-8-46.
49. Qian XH, Song XX, Liu XL, Chen SD, Tang HD. Inflammatory pathways in Alzheimer's disease mediated by gut microbiota. *Ageing Res. Rev.* **2021**, *68*, 101317, doi:10.1016/j.arr.2021.101317.
50. James, A.P.; Pal, S.; Gennat, H.C.; Vine, D.F.; Mamo, J.C.L. The incorporation and metabolism of amyloid-β into chylomicron-like lipid emulsions. *J. Alzheimer's Dis.* **2003**, *5*, 179–188, doi:10.3233/JAD-2003-5302.
51. Kim, S.; Kwon, S.H.; Kam, T.I.; Panicker, N.; Karuppagounder, S.S.; Lee, S.; Lee, J.H.; Kim, W.R.; Kook, M.; Foss, C.A.; et al. Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron* **2019**, *103*, 627-641.e7, doi:10.1016/j.neuron.2019.05.035.
52. Sgritta, M.; Dooling, S.W.; Buffington, S.A.; Momin, E.N.; Francis, M.B.; Britton, R.A.; Costa-Mattioli, M. Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron* **2019**, *101*, 246-259.e6, doi:10.1016/j.neuron.2018.11.018.
53. Batista, C.R.A.; Gomes, G.F.; Candelario-Jalil, E.; Fiebich, B.L.; de Oliveira, A.C.P. Lipopolysaccharide-induced neuroinflammation as a bridge to understand neurodegeneration. *Int. J. Mol. Sci.* **2019**, *20*, 2293, doi:10.3390/ijms20092293.
54. Wang, X.; Sun, G.; Feng, T.; Zhang, J.; Huang, X.; Wang, T.; Xie, Z.; Chu, X.; Yang, J.; Wang, H.; et al. Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. *Cell Res.* **2019**, *29*, 787–803, doi:10.1038/s41422-019-0216-x.
55. Xiao, S.; Chan, P.; Wang, T.; Hong, Z.; Wang, S.; Kuang, W.; He, J.; Pan, X.; Zhou, Y.; Ji, Y.; et al. A 36-week multicenter, randomized, double-blind, placebo-controlled, parallel-group, phase 3 clinical trial of sodium oligomannate for mild-to-moderate Alzheimer's dementia. *Alzheimer's Res. Ther.* **2021**, *13*, 62, doi:10.1186/s13195-021-00795-7.
56. Liddelow, S.A.; Guttenplan, K.A.; Clarke, L.E.; Bennett, F.C.; Bohlen, C.J.; Schirmer, L.; Bennett, M.L.; Münch, A.E.; Chung, W.S.; Peterson, T.C.; et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **2017**, *541*, 481–487, doi:10.1038/nature21029.
57. Allen, N.J.; Barres, B.A. Neuroscience: Glia - more than just brain glue. *Nature* **2009**, *457*, 675–677, doi: 10.1038/457675a.
58. Verkhratsky, A.; Nedergaard, M. Physiology of Astroglia. *Physiol. Rev.* **2018**, *98*, 239–389, doi:10.1152/physrev.00042.2016.
59. Pfrieger, F.W. Roles of glial cells in synapse development. *Cell. Mol. Life Sci.* **2009**, *66*, 2037–2047, doi: 10.1007/s00018-009-0005-7.
60. Heneka, M.T.; Rodríguez, J.J.; Verkhratsky, A. Neuroglia in neurodegeneration. *Brain Res. Rev.* **2010**, *63*, 189–211, doi: 10.1016/j.brainresrev.2009.11.004.
61. Sofroniew, M. V; Vinters, H. V Astrocytes: biology and pathology. *Acta Neuropathol.* **2010**, *119*, 7–35, doi:10.1007/s00401-009-0619-8.
62. Verkhratsky, A.; Parpura, V.; Rodríguez, J.J. Where the thoughts dwell: The physiology of neuronal-glial "diffuse neural net". *Brain Res. Rev.* **2011**, *66*, 133–151, doi: 10.1016/j.brainresrev.2010.05.002.
63. Macvicar, B.A.; Newman, E.A. Astrocyte regulation of blood flow in the brain. *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, 1–15, doi:10.1101/cshperspect.a020388.
64. Govindpani, K.; McNamara, L.G.; Smith, N.R.; Vinnakota, C.; Waldvogel, H.J.; Faull, R.L.; Kwakowsky, A. Vascular Dysfunction in Alzheimer's Disease: A Prelude to the Pathological Process or a Consequence of It? *J. Clin. Med.* **2019**, *8*, 651, doi:10.3390/jcm8050651.
65. Dudvarski Stankovic, N.; Teodorczyk, M.; Ploen, R.; Zipp, F.; Schmidt, M.H.H. Microglia–blood vessel interactions: a double-edged sword in brain pathologies. *Acta Neuropathol.* **2016**, *131*, 347–363, doi: 10.1007/s00401-015-1524-y.
66. Iadecola, C. The Pathobiology of Vascular Dementia. *Neuron* **2013**, *80*, 844–866, doi: 10.1016/j.neuron.2013.10.008.
67. Wang, H.; Golob, E.J.; Su, M.Y. Vascular volume and blood-brain barrier permeability measured by dynamic contrast enhanced MRI in hippocampus and cerebellum of patients with MCI and normal controls. *J. Magn. Reson. Imaging* **2006**, *24*,

695–700, doi:10.1002/jmri.20669.

68. Starr, J.M.; Farrall, A.J.; Armitage, P.; McGurn, B.; Wardlaw, J. Blood-brain barrier permeability in Alzheimer's disease: a case-control MRI study. *Psychiatry Res. - Neuroimaging* **2009**, *171*, 232–241, doi:10.1016/j.pscychresns.2008.04.003.
69. Iadecola, C.; Zhang, F.; Niwa, K.; Eckman, C.; Turner, S.K.; Fischer, E.; Younkin, S.; Borchelt, D.R.; Hsiao, K.K.; Carlson, G.A. SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein. *Nat. Neurosci.* **1999**, *2*, 157–161, doi:10.1038/5715.
70. Iadecola, C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat. Rev. Neurosci.* **2004**, *5*, 347–360, doi: 10.1038/nrn1387.
71. Kelly, P.; Denver, P.; Satchell, S.C.; Ackermann, M.; Konerding, M.A.; Mitchell, C.A. Microvascular ultrastructural changes precede cognitive impairment in the murine APPswe/PS1dE9 model of Alzheimer's disease. *Angiogenesis* **2017**, *20*, 567–580, doi:10.1007/s10456-017-9568-3.
72. Abbott, N.J. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J. Anat.* **2002**, *200*, 629–638, doi: 10.1046/j.1469-7580.2002.00064.x.
73. Iturria-Medina, Y.; Sotero, R.C.; Toussaint, P.J.; Mateos-Pérez, J.M.; Evans, A.C.; Weiner, M.W.; Aisen, P.; Petersen, R.; Jack, C.R.; Jagust, W.; et al. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat. Commun.* **2016**, *7*, doi:10.1038/ncomms11934.
74. Sweeney, M.D.; Sagare, A.P.; Zlokovic, B.V. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat. Rev. Neurol.* **2018**, *14*, 133–150, doi: 10.1038/nrneurol.2017.188.
75. Kress, B.T.; Iliff, J.J.; Xia, M.; Wang, M.; Wei Bs, H.S.; Zeppenfeld, D.; Xie, L.; Hongyi Kang, B.S.; Xu, Q.; Liew, J.A.; et al. Impairment of paravascular clearance pathways in the aging brain. *Ann. Neurol.* **2014**, *76*, 845–861, doi:10.1002/ana.24271.
76. Friede, R.L.; van Houten, W.H. Relations between post-mortem alterations and glycolytic metabolism in the brain. *Exp. Neurol.* **1961**, *4*, 197–204, doi:10.1016/0014-4886(61)90041-3.
77. Hulse, R.E.; Winterfield, J.; Kunkler, P.; Kraig, R.P. Astrocytic Clasmatodendrosis in Hippocampal Organ Culture. *Glia* **2001**, *33*, 169–179, doi:10.1002/1098-1136(200102)33:2<169::AID-GLIA1016>3.0.CO;2-B.
78. Jiang, X.; Andjelkovic, A. V; Zhu, L.; Yang, T.; Bennett, M.V.L.; Chen, J.; Keep, R.F.; Shi, Y. Blood-brain barrier dysfunction and recovery after ischemic stroke. *Prog. Neurobiol.* **2018**, *163–164*, 144–171, doi: 10.1016/j.pneurobio.2017.10.001.
79. Chen, A.; Akinyemi, R.O.; Hase, Y.; Firbank, M.J.; Ndung'u, M.N.; Foster, V.; Craggs, L.J.L.; Washida, K.; Okamoto, Y.; Thomas, A.J.; et al. Frontal White Matter Hyperintensities, Clasmatodendrosis and Gliovascular Abnormalities in Ageing and Post-Stroke Dementia - PubMed. *Brain* **2016**, *139*, 242–258, doi:10.1093/brain/awv328.
80. Cerbai, F.; Lana, D.; Nosi, D.; Petkova-Kirova, P.; Zecchi, S.; Brothers, H.M.; Wenk, G.L.; Giovannini, M.G. The Neuron-Astrocyte-Microglia Triad in Normal Brain Ageing and in a Model of Neuroinflammation in the Rat Hippocampus. *PLoS One* **2012**, *7*, doi:10.1371/journal.pone.0045250.
81. Nagele, R.G.; Wegiel, J.; Venkataraman, V.; Imaki, H.; Wang, K.C.; Wegiel, J. Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. *Neurobiol. Aging* **2004**, *25*, 663–674, doi:10.1016/j.neurobiolaging.2004.01.007.
82. Mercatelli, R.; Lana, D.; Bucciantini, M.; Giovannini, M.G.; Cerbai, F.; Quercioli, F.; Zecchi-Orlandini, S.; Delfino, G.; Wenk, G.L.; Nosi, D. Clasmatodendrosis and b-amyloidosis in aging hippocampus. *FASEB J.* **2016**, *30*, 1480–1491, doi:10.1096/fj.15-275503.
83. Marques, F.; Sousa, J.C.; Sousa, N.; Palha, J.A. Blood-brain-barriers in aging and in Alzheimer's disease. *Mol. Neurodegener.* **2013**, *8*, 38, doi: 10.1186/1750-1326-8-38.
84. Frenkel, D.; Wilkinson, K.; Zhao, L.; Hickman, S.E.; Means, T.K.; Puckett, L.; Farfara, D.; Kingery, N.D.; Weiner, H.L.; El Khoury, J. Scara1 deficiency impairs clearance of soluble amyloid-β by mononuclear phagocytes and accelerates Alzheimer's-like disease progression. *Nat. Commun.* **2013**, *4*, 2030, doi:10.1038/ncomms3030.
85. Hayes, C.L.; Dong, J.; Galipeau, H.J.; Jury, J.; McCarville, J.; Huang, X.; Wang, X.Y.; Naidoo, A.; Anbazhagan, A.N.; Libertucci,

J.; et al. Commensal microbiota induces colonic barrier structure and functions that contribute to homeostasis. *Sci. Rep.* **2018**, *8*, 14184, doi:10.1038/s41598-018-32366-6.

86. Braniste, V.; Al-Asmakh, M.; Kowal, C.; Anuar, F.; Abbaspour, A.; Tóth, M.; Korecka, A.; Bakocevic, N.; Guan, N.L.; Kundu, P.; et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* **2014**, *6*, 263ra158, doi:10.1126/scitranslmed.3009759.
87. Hoyles, L.; Snelling, T.; Umlai, U.K.; Nicholson, J.K.; Carding, S.R.; Glen, R.C.; McArthur, S. Microbiome–host systems interactions: Protective effects of propionate upon the blood–brain barrier. *Microbiome* **2018**, *6*, 55, doi:10.1186/s40168-018-0439-y.
88. Rothhammer, V.; Mascanfroni, I.D.; Bunse, L.; Takenaka, M.C.; Kenison, J.E.; Mayo, L.; Chao, C.C.; Patel, B.; Yan, R.; Blain, M.; et al. Type i interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* **2016**, *22*, 586–597, doi:10.1038/nm.4106.
89. Zamanian, J.L.; Xu, L.; Foo, L.C.; Nouri, N.; Zhou, L.; Giffard, R.G.; Barres, B.A. Genomic analysis of reactive astrogliosis. *J. Neurosci.* **2012**, *32*, 6391–6410, doi:10.1523/JNEUROSCI.6221-11.2012.
90. Nau, R.; Sörgel, F.; Eiffert, H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin. Microbiol. Rev.* **2010**, *23*, 858–883, doi:10.1128/CMR.00007-10.
91. Daneman, R.; Zhou, L.; Kebede, A.A.; Barres, B.A. Pericytes are required for blood–brain barrier integrity during embryogenesis. *Nature* **2010**, *468*, 562–566, doi:10.1038/nature09513.
92. Hagan, N.; Ben-Zvi, A. The molecular, cellular, and morphological components of blood-brain barrier development during embryogenesis. *Semin. Cell Dev. Biol.* **2015**, *38*, 7–15, doi: 10.1016/j.semcdb.2014.12.006.
93. Fröhlich, E.E.; Farzi, A.; Mayerhofer, R.; Reichmann, F.; Jačan, A.; Wagner, B.; Zinser, E.; Bordag, N.; Magnes, C.; Fröhlich, E.; et al. Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain. Behav. Immun.* **2016**, *56*, 140–155, doi:10.1016/j.bbi.2016.02.020.
94. Zhu, X.; Smith, M.A.; Honda, K.; Aliev, G.; Moreira, P.I.; Nunomura, A.; Casadesus, G.; Harris, P.L.R.; Siedlak, S.L.; Perry, G. Vascular oxidative stress in Alzheimer disease. *J. Neurol. Sci.* **2007**, *257*, 240–246, doi:10.1016/j.jns.2007.01.039.
95. Miyazaki, K.; Ohta, Y.; Nagai, M.; Morimoto, N.; Kurata, T.; Takehisa, Y.; Ikeda, Y.; Matsuura, T.; Abe, K. Disruption of neurovascular unit prior to motor neuron degeneration in amyotrophic lateral sclerosis. *J. Neurosci. Res.* **2011**, *89*, 718–728, doi:10.1002/jnr.22594.
96. Freeman, L.R.; Keller, J.N. Oxidative stress and cerebral endothelial cells: Regulation of the blood-brain-barrier and antioxidant based interventions. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2012**, *1822*, 822–829, doi: 10.1016/j.bbadis.2011.12.009.
97. Guan, J.; Pavlovic, D.; Dalkie, N.; Waldvogel, H.J.; O'Carroll, S.J.; Green, C.R.; Nicholson, L.F.B. Vascular degeneration in parkinsons disease. *Brain Pathol.* **2013**, *23*, 154–164, doi:10.1111/j.1750-3639.2012.00628.x.
98. Sweeney, M.D.; Kisler, K.; Montagne, A.; Toga, A.W.; Zlokovic, B. V. The role of brain vasculature in neurodegenerative disorders. *Nat. Neurosci.* **2018**, *21*, 1318–1331, doi: 10.1038/s41593-018-0234-x.
99. Wang, M.; Ding, F.; Deng, S.Y.; Guo, X.; Wang, W.; Iliff, J.J.; Nedergaard, M. Focal solute trapping and global glymphatic pathway impairment in a murine model of multiple microinfarcts. *J. Neurosci.* **2017**, *37*, 2870–2877, doi:10.1523/JNEUROSCI.2112-16.2017.
100. Ries, M.; Sastre, M. Mechanisms of Aβ clearance and degradation by glial cells. *Front. Aging Neurosci.* **2016**, *8*, 160, doi: 10.3389/fnagi.2016.00160.
101. Xu, Z.; Xiao, N.; Chen, Y.; Huang, H.; Marshall, C.; Gao, J.; Cai, Z.; Wu, T.; Hu, G.; Xiao, M. Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain Aβ accumulation and memory deficits. *Mol. Neurodegener.* **2015**, *10*, 58, doi:10.1186/s13024-015-0056-1.
102. Wikoff, W.R.; Anfora, A.T.; Liu, J.; Schultz, P.G.; Lesley, S.A.; Peters, E.C.; Siuzdak, G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 3698–3703, doi:10.1073/pnas.0812874106.

103. Boillot, A.; Demmer, R.T.; Mallat, Z.; Sacco, R.L.; Jacobs, D.R.; Benessiano, J.; Tedgui, A.; Rundek, T.; Papapanou, P.N.; Desvarieux, M. Periodontal microbiota and phospholipases: The Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Atherosclerosis* **2015**, *242*, 418–423, doi:10.1016/j.atherosclerosis.2015.07.039.
104. Zhang, J.; Yu, C.; Zhang, X.; Chen, H.; Dong, J.; Lu, W.; Song, Z.; Zhou, W. Porphyromonas gingivalis lipopolysaccharide induces cognitive dysfunction, mediated by neuronal inflammation via activation of the TLR4 signaling pathway in C57BL/6 mice. *J. Neuroinflammation* **2018**, *15*, 37, doi:10.1186/s12974-017-1052-x.
105. Boche, D.; Perry, V.H.; Nicoll, J.A.R. Review: Activation patterns of microglia and their identification in the human brain. *Neuropathol. Appl. Neurobiol.* **2013**, *39*, 3–18, doi: 10.1111/nan.12011.
106. Fetler, L.; Amigorena, S. Brain under surveillance: The microglia patrol. *Science* **2005**, *309*, 392–393, doi: 10.1126/science.1114852.
107. Kettenmann, H.; Kirchhoff, F.; Verkhratsky, A. Microglia: New Roles for the Synaptic Stripper. *Neuron* **2013**, *77*, 10–18, doi: 10.1016/j.neuron.2012.12.023.
108. Schafer, D.P.; Stevens, B. Phagocytic glial cells: Sculpting synaptic circuits in the developing nervous system. *Curr. Opin. Neurobiol.* **2013**, *23*, 1034–1040, doi: 10.1016/j.conb.2013.09.012.
109. Paolicelli, R.C.; Bolasco, G.; Pagani, F.; Maggi, L.; Scianni, M.; Panzanelli, P.; Giustetto, M.; Ferreira, T.A.; Guiducci, E.; Dumas, L.; et al. Synaptic pruning by microglia is necessary for normal brain development. *Science* **2011**, *333*, 1456–1458, doi:10.1126/science.1202529.
110. Wake, H.; Moorhouse, A.J.; Miyamoto, A.; Nabekura, J. Microglia: Actively surveying and shaping neuronal circuit structure and function. *Trends Neurosci.* **2013**, *36*, 209–217, doi: 10.1016/j.tins.2012.11.007.
111. Parkhurst, C.N.; Yang, G.; Ninan, I.; Savas, J.N.; Yates, J.R.; Lafaille, J.J.; Hempstead, B.L.; Littman, D.R.; Gan, W.B. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* **2013**, *155*, 1596–1609, doi:10.1016/j.cell.2013.11.030.
112. Schafer, D.P.; Lehrman, E.K.; Kautzman, A.G.; Koyama, R.; Mardinly, A.R.; Yamasaki, R.; Ransohoff, R.M.; Greenberg, M.E.; Barres, B.A.; Stevens, B. Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron* **2012**, *74*, 691–705, doi:10.1016/j.neuron.2012.03.026.
113. Fitzner, D.; Schnaars, M.; Van Rossum, D.; Krishnamoorthy, G.; Dibaj, P.; Bakhti, M.; Regen, T.; Hanisch, U.K.; Simons, M. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J. Cell Sci.* **2011**, *124*, 447–458, doi:10.1242/jcs.074088.
114. Safaiyan, S.; Kannaiyan, N.; Snaidero, N.; Brioschi, S.; Biber, K.; Yona, S.; Edinger, A.L.; Jung, S.; Rossner, M.J.; Simons, M. Age-related myelin degradation burdens the clearance function of microglia during aging. *Nat. Neurosci.* **2016**, *19*, 995–998, doi:10.1038/nn.4325.
115. Rothhammer, V.; Borucki, D.M.; Tjon, E.C.; Takenaka, M.C.; Chao, C.C.; Ardura-Fabregat, A.; De Lima, K.A.; Gutiérrez-Vázquez, C.; Hewson, P.; Staszewski, O.; et al. Microglial control of astrocytes in response to microbial metabolites. *Nature* **2018**, *557*, 724–728, doi:10.1038/s41586-018-0119-x.
116. Prinz, M.; Priller, J.; Sisodia, S.S.; Ransohoff, R.M. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* **2011**, *14*, 1227–1235, doi: 10.1038/nn.2923.
117. Prinz, M.; Priller, J. Microglia and brain macrophages in the molecular age: From origin to neuropsychiatric disease. *Nat. Rev. Neurosci.* **2014**, *15*, 300–312, doi: 10.1038/nrn3722.
118. Hanisch, U.K.; Kettenmann, H. Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* **2007**, *10*, 1387–1394, doi: 10.1038/nn1997.
119. Henneman, W.J.P.; Sluimer, J.D.; Barnes, J.; Van Der Flier, W.M.; Sluimer, I.C.; Fox, N.C.; Scheltens, P.; Vrenken, H.; Barkhof, F. Hippocampal atrophy rates in Alzheimer disease: Added value over whole brain volume measures. *Neurology* **2009**, *72*, 999–1007, doi:10.1212/01.wnl.0000344568.09360.31.

120. Cagnin, A.; Rossor, M.; Sampson, E.L.; MacKinnon, T.; Banati, R.B. In vivo detection of microglial activation in frontotemporal dementia. *Ann. Neurol.* **2004**, *56*, 894–897, doi:10.1002/ana.20332.
121. Rosso, S.M.; Landweer, E.J.; Houterman, M.; Donker Kaat, L.; Van Duijn, C.M.; Van Swieten, J.C. Medical and environmental risk factors for sporadic frontotemporal dementia: A retrospective case-control study. *J. Neurol. Neurosurg. Psychiatry* **2003**, *74*, 1574–1576, doi:10.1136/jnnp.74.11.1574.
122. Perry, V.H. Innate inflammation in Parkinson's disease. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, doi:10.1101/cshperspect.a009373.
123. Block, M.L.; Zecca, L.; Hong, J.S. Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* **2007**, *8*, 57–69, doi:10.1038/nrn2038.
124. Koenigsknecht-Talboo, J.; Landreth, G.E. Microglial phagocytosis induced by fibrillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines. *J. Neurosci.* **2005**, *25*, 8240–8249, doi:10.1523/JNEUROSCI.1808-05.2005.
125. Blume, T.; Focke, C.; Peters, F.; Deussing, M.; Albert, N.L.; Lindner, S.; Gildehaus, F.J.; Von Ungern-Sternberg, B.; Ozmen, L.; Baumann, K.; et al. Microglial response to increasing amyloid load saturates with aging: A longitudinal dual tracer in vivo μpET-study 11 Medical and Health Sciences 1109 Neurosciences. *J. Neuroinflammation* **2018**, *15*, 307, doi:10.1186/s12974-018-1347-6.
126. Davalos, D.; Grutzendler, J.; Yang, G.; Kim, J. V; Zuo, Y.; Jung, S.; Littman, D.R.; Dustin, M.L.; Gan, W.B. ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* **2005**, *8*, 752–758, doi:10.1038/nn1472.
127. Nimmerjahn, A.; Kirchhoff, F.; Helmchen, F. Neuroscience: Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science.* **2005**, *308*, 1314–1318, doi:10.1126/science.1110647.
128. Erny, D.; De Angelis, A.L.H.; Jaitin, D.; Wieghofer, P.; Staszewski, O.; David, E.; Keren-Shaul, H.; Mahlakoiv, T.; Jakobshagen, K.; Buch, T.; et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* **2015**, *18*, 965–977, doi:10.1038/nn.4030.
129. Sampson, T.R.; Debelius, J.W.; Thron, T.; Janssen, S.; Shastri, G.G.; Ilhan, Z.E.; Challis, C.; Schretter, C.E.; Rocha, S.; Gradinaru, V.; et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* **2016**, *167*, 1469-1480.e12, doi:10.1016/j.cell.2016.11.018.
130. Thion, M.S.; Low, D.; Silvin, A.; Chen, J.; Grisel, P.; Schulte-Schrepping, J.; Blecher, R.; Ulas, T.; Squarzoni, P.; Hoeffel, G.; et al. Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell* **2018**, *172*, 500-516.e16, doi:10.1016/j.cell.2017.11.042.
131. Chu, C.; Murdock, M.H.; Jing, D.; Won, T.H.; Chung, H.; Kressel, A.M.; Tsaava, T.; Addorisio, M.E.; Putzel, G.G.; Zhou, L.; et al. The microbiota regulate neuronal function and fear extinction learning. *Nature* **2019**, *574*, 543–548, doi:10.1038/s41586-019-1644-y.
132. Cunningham, C.; Wilcockson, D.C.; Campion, S.; Lunnon, K.; Perry, V.H. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J. Neurosci.* **2005**, *25*, 9275–9284, doi:10.1523/JNEUROSCI.2614-05.2005.
133. Holmes, C.; El-Okl, M.; Williams, A.L.; Cunningham, C.; Wilcockson, D.; Perry, V.H. Systemic infection, interleukin 1β, and cognitive decline in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **2003**, *74*, 788–789, doi:10.1136/jnnp.74.6.788.
134. Sierra, A.; de Castro, F.; del Río-Hortega, J.; Rafael Iglesias-Rozas, J.; Garrosa, M.; Kettenmann, H. The "Big-Bang" for modern glial biology: Translation and comments on Pío del Río-Hortega 1919 series of papers on microglia. *Glia* **2016**, *64*, 1801–1840, doi:10.1002/glia.23046.
135. Guzman-Martinez, L.; Maccioni, R.B.; Andrade, V.; Navarrete, L.P.; Pastor, M.G.; Ramos-Escobar, N. Neuroinflammation as a common feature of neurodegenerative disorders. *Front. Pharmacol.* **2019**, *10*, 1008, doi: 10.3389/fphar.2019.01008.
136. Loane, D.J.; Kumar, A. Microglia in the TBI brain: The good, the bad, and the dysregulated. *Exp. Neurol.* **2016**, *275*, 316–327, doi: 10.1016/j.expneurol.2015.08.018.

137. Hu, X.; Li, P.; Guo, Y.; Wang, H.; Leak, R.K.; Chen, S.; Gao, Y.; Chen, J. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* **2012**, *43*, 3063–3070, doi:10.1161/STROKEAHA.112.659656.
138. Suenaga, J.; Hu, X.; Pu, H.; Shi, Y.; Hassan, S.H.; Xu, M.; Leak, R.K.; Stetler, R.A.; Gao, Y.; Chen, J. White matter injury and microglia/macrophage polarization are strongly linked with age-related long-term deficits in neurological function after stroke. *Exp. Neurol.* **2015**, *272*, 109–119, doi:10.1016/j.expneurol.2015.03.021.
139. Arcuri, C.; Mecca, C.; Bianchi, R.; Giambanco, I.; Donato, R. The pathophysiological role of microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing CNS. *Front. Mol. Neurosci.* **2017**, *10*, 191, doi: 10.3389/fnmol.2017.00191.
140. Gordon, S.; Plüddemann, A. Macrophage clearance of apoptotic cells: A critical assessment. *Front. Immunol.* **2018**, *9*, 127, doi: 10.3389/fimmu.2018.00127.
141. Mantovani, A.; Sica, A.; Sozzani, S.; Allavena, P.; Vecchi, A.; Locati, M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **2004**, *25*, 677–686, doi: 10.1016/j.it.2004.09.015.
142. Böttcher, C.; Schlickeiser, S.; Sneeboer, M.A.M.; Kunkel, D.; Knop, A.; Paza, E.; Fidzinski, P.; Kraus, L.; Snijders, G.J.L.; Kahn, R.S.; et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat. Neurosci.* **2019**, *22*, 78–90, doi:10.1038/s41593-018-0290-2.
143. Masuda, T.; Sankowski, R.; Staszewski, O.; Böttcher, C.; Amann, L.; Sagar; Scheiwe, C.; Nessler, S.; Kunz, P.; van Loo, G.; et al. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* **2019**, *566*, 388–392, doi:10.1038/s41586-019-0924-x.
144. Grabert, K.; Michoel, T.; Karavolos, M.H.; Clohisey, S.; Kenneth Baillie, J.; Stevens, M.P.; Freeman, T.C.; Summers, K.M.; McColl, B.W. Microglial brain regionâ 'dependent diversity and selective regional sensitivities to aging. *Nat. Neurosci.* **2016**, *19*, 504–516, doi:10.1038/nn.4222.
145. Hart, A.D.; Wyttenbach, A.; Hugh Perry, V.; Teeling, J.L. Age related changes in microglial phenotype vary between CNS regions: Grey versus white matter differences. *Brain. Behav. Immun.* **2012**, *26*, 754–765, doi:10.1016/j.bbi.2011.11.006.
146. de Haas, A.H.; Boddeke, H.W.G.M.; Biber, K. Region-specific expression of immunoregulatory proteins on microglia in the healthy CNS. *Glia* **2008**, *56*, 888–894, doi:10.1002/glia.20663.
147. Rappert, A.; Bechmann, I.; Pivneva, T.; Mahlo, J.; Biber, K.; Nolte, C.; Kovac, A.D.; Gerard, C.; Boddeke, H.W.G.M.; Nitsch, R.; et al. CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion. *J. Neurosci.* **2004**, *24*, 8500–8509, doi:10.1523/JNEUROSCI.2451-04.2004.
148. Krauthausen, M.; Saxe, S.; Zimmermann, J.; Emrich, M.; Heneka, M.T.; Müller, M. CXCR3 modulates glial accumulation and activation in cuprizone-induced demyelination of the central nervous system. *J. Neuroinflammation* **2014**, *11*, 109, doi:10.1186/1742-2094-11-109.
149. Bonham, L.W.; Sirkis, D.W.; Yokoyama, J.S. The transcriptional landscape of microglial genes in aging and neurodegenerative disease. *Front. Immunol.* **2019**, *10*, 1170, doi:10.3389/fimmu.2019.01170.
150. Pandey, S.; Kawai, T.; Akira, S. Microbial sensing by toll-like receptors and intracellular nucleic acid sensors. *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, a016246, doi:10.1101/cshperspect.a016246.
151. Banks, W.A.; Robinson, S.M. Minimal penetration of lipopolysaccharide across the murine blood-brain barrier. *Brain. Behav. Immun.* **2010**, *24*, 102–109, doi:10.1016/j.bbi.2009.09.001.
152. Vargas-Caraveo, A.; Sayd, A.; Maus, S.R.; Caso, J.R.; Madrigal, J.L.M.; García-Bueno, B.; Leza, J.C. Lipopolysaccharide enters the rat brain by a lipoprotein-mediated transport mechanism in physiological conditions. *Sci. Rep.* **2017**, *7*, 13113, doi:10.1038/s41598-017-13302-6.
153. Zhang, X.; Dong, H.; Zhang, S.; Lu, S.; Sun, J.; Qian, Y. Enhancement of LPS-induced microglial inflammation response via TLR4 under high glucose conditions. *Cell. Physiol. Biochem.* **2015**, *35*, 1571–1581, doi:10.1159/000373972.

154. Dansokho, C.; Heneka, M.T. Neuroinflammatory responses in Alzheimer's disease. *J. Neural Transm.* 2018, *125*, 771–779, doi: 10.1007/s00702-017-1831-7.
155. Faulkner, J.R.; Herrmann, J.E.; Woo, M.J.; Tansey, K.E.; Doan, N.B.; Sofroniew, M. V Reactive Astrocytes Protect Tissue and Preserve Function after Spinal Cord Injury. *J. Neurosci.* **2004**, *24*, 2143–2155, doi:10.1523/JNEUROSCI.3547-03.2004.
156. Li, L.; Lundkvist, A.; Andersson, D.; Wilhelmsson, U.; Nagai, N.; Pardo, A.C.; Nodin, C.; Ståhlberg, A.; Aprico, K.; Larsson, K.; et al. Protective role of reactive astrocytes in brain ischemia. *J. Cereb. Blood Flow Metab.* **2008**, *28*, 468–481, doi:10.1038/sj.jcbfm.9600546.
157. Myer, D.J.; Gurkoff, G.G.; Lee, S.M.; Hovda, D.A.; Sofroniew, M. V Essential Protective Roles of Reactive Astrocytes in Traumatic Brain Injury - PubMed. *Brain* **2006**, *129*, 2761–2772, doi:10.1093/brain/awl165.
158. Sierra, A.; Abiega, O.; Shahraz, A.; Neumann, H. Janus-faced microglia: Beneficial and detrimental consequences of microglial phagocytosis. *Front. Cell. Neurosci.* 2013, *7*, 6. doi: 10.3389/fncel.2013.00006.
159. Brown, G.C.; Neher, J.J. Microglial phagocytosis of live neurons. *Nat. Rev. Neurosci.* **2014**, *15*, 209–216, doi:10.1038/nrn3710.
160. Vilalta, A.; Brown, G.C. Neurophagy, the phagocytosis of live neurons and synapses by glia, contributes to brain development and disease. *FEBS J.* **2018**, *285*, 3566–3575, doi: 10.1111/febs.14323.
161. Neher, J.J.; Neniskyte, U.; Zhao, J.-W.; Bal-Price, A.; Tolkovsky, A.M.; Brown, G.C. Inhibition of Microglial Phagocytosis Is Sufficient To Prevent Inflammatory Neuronal Death. *J. Immunol.* **2011**, *186*, 4973–4983, doi:10.4049/jimmunol.1003600.
162. Neher, J.J.; Neniskyte, U.; Brown, G.C. Primary phagocytosis of neurons by inflamed microglia: Potential roles in neurodegeneration. *Front. Pharmacol.* **2012**, *3*, 27, doi:10.3389/fphar.2012.00027.
163. Giunta, B.; Fernandez, F.; Nikolic, W. V; Obregon, D.; Rrapo, E.; Town, T.; Tan, J. Inflammaging as a prodrome to Alzheimer's disease. *J. Neuroinflammation* **2008**, *5*, 51, doi: 10.1186/1742-2094-5-51.
164. Deleidi, M.; Jäggle, M.; Rubino, G. Immune ageing, dysmetabolism and inflammation in neurological diseases. *Front. Neurosci.* **2015**, *9*, 172, doi:10.3389/fnins.2015.00172.
165. Badimon, A.; Strasburger, H.J.; Ayata, P.; Chen, X.; Nair, A.; Ikegami, A.; Hwang, P.; Chan, A.T.; Graves, S.M.; Uweru, J.O.; et al. Negative feedback control of neuronal activity by microglia. *Nature* **2020**, *586*, 417–423, doi:10.1038/s41586-020-2777-8.
166. Li, Y.; Du, X.F.; Liu, C.S.; Wen, Z.L.; Du, J.L. Reciprocal Regulation between Resting Microglial Dynamics and Neuronal Activity In Vivo. *Dev. Cell* **2012**, *23*, 1189–1202, doi:10.1016/j.devcel.2012.10.027.
167. Akiyoshi, R.; Wake, H.; Kato, D.; Horiuchi, H.; Ono, R.; Ikegami, A.; Haruwaka, K.; Omori, T.; Tachibana, Y.; Moorhouse, A.J.; et al. Microglia enhance synapse activity to promote local network synchronization. *eNeuro* **2018**, *5*, ENEURO.0088-18.2018, doi:10.1523/ENEURO.0088-18.2018.
168. Kato, G.; Inada, H.; Wake, H.; Akiyoshi, R.; Miyamoto, A.; Eto, K.; Ishikawa, T.; Moorhouse, A.J.; Strassman, A.M.; Nabekura, J. Microglial contact prevents excess depolarization and rescues neurons from excitotoxicity. *eNeuro* **2016**, *3*, 9133–9144, doi:10.1523/ENEURO.0004-16.2016.
169. Wake, H.; Moorhouse, A.J.; Jinno, S.; Kohsaka, S.; Nabekura, J. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J. Neurosci.* **2009**, *29*, 3974–3980, doi:10.1523/JNEUROSCI.4363-08.2009.
170. Cserép, C.; Pósfai, B.; Lénárt, N.; Fekete, R.; László, Z.I.; Lele, Z.; Orsolits, B.; Molnár, G.; Heindl, S.; Schwarcz, A.D.; et al. Microglia monitor and protect neuronal function through specialized somatic purinergic junctions. *Science* **2020**, *367*, 528–537, doi:10.1126/science.aax6752.
171. Palop, J.J.; Chin, J.; Roberson, E.D.; Wang, J.; Thwin, M.T.; Bien-Ly, N.; Yoo, J.; Ho, K.O.; Yu, G.Q.; Kreitzer, A.; et al. Aberrant Excitatory Neuronal Activity and Compensatory Remodeling of Inhibitory Hippocampal Circuits in Mouse Models of Alzheimer's Disease. *Neuron* **2007**, *55*, 697–711, doi:10.1016/j.neuron.2007.07.025.
172. Lam, A.D.; Deck, G.; Goldman, A.; Eskandar, E.N.; Noebels, J.; Cole, A.J. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat. Med.* **2017**, *23*, 678–680, doi:10.1038/nm.4330.

173. Krasemann, S.; Madore, C.; Cialic, R.; Baufeld, C.; Calcagno, N.; El Fatimy, R.; Beckers, L.; O'Loughlin, E.; Xu, Y.; Fanek, Z.; et al. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* **2017**, *47*, 566-581.e9, doi:10.1016/j.immuni.2017.08.008.
174. Mildner, A.; Huang, H.; Radke, J.; Stenzel, W.; Priller, J. P2Y12 receptor is expressed on human microglia under physiological conditions throughout development and is sensitive to neuroinflammatory diseases. *Glia* **2017**, *65*, 375–387, doi:10.1002/glia.23097.
175. Abdel-Haq, R.; Schlachetzki, J.C.M.; Glass, C.K.; Mazmanian, S.K. Microbiome–microglia connections via the gut–brain axis. *J. Exp. Med.* **2019**, *216*, 41–59, doi:10.1084/jem.20180794.
176. Bonaz, B.; Bazin, T.; Pellissier, S. The vagus nerve at the interface of the microbiota-gut-brain axis. *Front. Neurosci.* **2018**, *12*, 49, doi: 10.3389/fnins.2018.00049.
177. Gosselin, D.; Link, V.M.; Romanoski, C.E.; Fonseca, G.J.; Eichenfield, D.Z.; Spann, N.J.; Stender, J.D.; Chun, H.B.; Garner, H.; Geissmann, F.; et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* **2014**, *159*, 1327–1340, doi:10.1016/j.cell.2014.11.023.
178. Lavin, Y.; Winter, D.; Blecher-Gonen, R.; David, E.; Keren-Shaul, H.; Merad, M.; Jung, S.; Amit, I. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* **2014**, *159*, 1312-1326, doi:10.1016/j.cell.2014.11.018.
179. Datta, M.; Staszewski, O.; Raschi, E.; Frosch, M.; Hagemeyer, N.; Tay, T.L.; Blank, T.; Kreutzfeldt, M.; Merkler, D.; Ziegler-Waldkirch, S.; et al. Histone Deacetylases 1 and 2 Regulate Microglia Function during Development, Homeostasis, and Neurodegeneration in a Context-Dependent Manner. *Immunity* **2018**, *48*, 514-529.e6, doi:10.1016/j.immuni.2018.02.016.
180. Sharma, M.; Li, Y.; Stoll, M.L.; Tollefsbol, T.O. The Epigenetic Connection Between the Gut Microbiome in Obesity and Diabetes. *Front. Genet.* **2020**, *10*, 1329, doi:10.3389/fgene.2019.01329.
181. Needham, B.D.; Trent, M.S. Fortifying the barrier: The impact of lipid A remodelling on bacterial pathogenesis. *Nat. Rev. Microbiol.* **2013**, *11*, 467–481, doi:10.1038/nrmicro3047.
182. Sender, R.; Fuchs, S.; Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol.* **2016**, *14*, 1–14, doi:10.1371/journal.pbio.1002533.
183. Hajjar, A.M.; Tsai, J.H.; Wilson, C.B.; Miller, S.I. Human Toll-like receptor 4 recognizes host-specific LPS modifications. *Nat. Immunol.* **2002**, *3*, 354–359, doi:10.1038/ni777.
184. Brown, G.C.; Neher, J.J. Eaten alive! Cell death by primary phagocytosis: "Phagoptosis". *Trends Biochem. Sci.* **2012**, *37*, 325–332, doi:10.1016/j.tibs.2012.05.002.
185. Wendeln, A.; Degenhardt, K.; Kaurani, L.; Gertig, M.; Ulas, T.; Jain, G.; Wagner, J.; Häsler, L.M.; Wild, K.; Skodras, A.; et al. Innate immune memory in the brain shapes neurological disease hallmarks. *Nature* **2018,** *556,* 332-338, doi: 10.1038/s41586-018-0023-4.

References

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